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INVESTIGATIONS
on
SERUM and INTESTINAL ALKALINE
PHOSPHATASE

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ABSTRACT

Rickets were produced in growing rats by diets low in calcium or by the addition of sodium oxalate or rhubarb to diets containing adequate amounts of calcium. Increased levels of serum alkaline phosphatase were observed with the development of rickets. The source of the increased serum alkaline phosphatase activity is probably the bone.

Rat intestinal alkaline phosphatase levels increase when fat is ingested. After prolonged fasting, however, the protein reserves of liver and intestine were depleted to such an extent that the animals could not maintain normal intestinal alkaline phosphatase activity when fat was ingested.

Force-feeding choline along with olive oil resulted in an increase of serum and intestinal alkaline phosphatase levels above those obtained with olive oil alone. Phospholipid synthesis, for which choline may be a limiting factor, appears to be a step in fat absorption from the intestine.

The ingestion of saturated and unsaturated long chain fatty acids and short chain fatty acids resulted in an elevation of both serum and intestinal alkaline phosphatase activity. Intestinal phosphatase would seem to play a role in the absorption of fatty acids.

INVESTIGATIONS
ON
SERUM AND INTESTINAL ALKALINE PHOSPHATASE

by
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GENERAL INTRODUCTION

The biocatalyst, phosphatase, which splits phosphate from phosphate esters has been a subject of research for the past fifty years. Early in the century phosphatase activity was linked with metabolism by Harden and Young (1) in their work on sugar fermentation. They found that inorganic phosphate actually combined with sugar as the first step in its degradation. The enzyme which hydrolyzed the resulting phosphate ester was given the name phosphatase.

Phosphatases, which may be either acid or alkaline, are classified under the general heading of esterases. Alkaline phosphatase refers to a specific esterase which catalyzes the hydrolysis of organic phosphomonoesters at a pH of about nine to ten. This enzyme acts on a great many substrates, for example; glycerophosphate, hexosephosphates, adenylic acid, and phenylphosphate.

Alkaline phosphatase is widely distributed in animal tissues and fluids, for example; in liver, bone, intestine, blood serum, kidney, and lung. Very high concentrations of the enzyme occur in intestinal mucosa, in ossifying cartilage, and in kidney cortex.

Kay(2) has presented evidence that esters of phosphoric acid are hydrolyzed in active tissue by one alkaline phosphatase. On the other hand, Cloetens (3) observed that two alkaline phosphatases are present in animal organs; a cyanide

sensitive enzyme and a cyanide insensitive enzyme. However recent evidence reveals that several alkaline phosphatases are present in animal tissues. Demsey and Deane (4) established the presence of various phosphatases in the duodenum of the mouse. These phosphatases exhibit variations in the degree of activity toward different substrates. Other investigators (5) have demonstrated that the alkaline phosphatase of liver, intestinal mucosa, bone, and kidney differ in their affinity for the same substrate.

According to Abul-Fadl and King (6), three constituents of alkaline phosphatase appear to be essential for its activity, namely; a specific protein apoenzyme, a specific dialysable group or coenzyme, and the magnesium ion. The three constituents were clearly demonstrated in their experiments using ox kidney phosphatase. They found that kidney phosphatase was inactivated during electrodialysis by the separation of an organic, possibly nitrogenous factor, which migrated to the cathode. This dialysable factor or coenzyme was shown to be essential for enzyme activity. Recent work by Sadasivan (7) has shown that both magnesium and zinc ions are important for the activity of the enzyme. He observed the effect of varying the relative proportions of magnesium and zinc and suggested the interesting theory that the relative proportions of the prosthetic groups of an enzyme determine the optimum pH of activity.

Gomori (8) has investigated intestinal alkaline phosphatase activity. He analysed mathematically the unusual time-hydrolysis curve of phosphatase activity in the absence of magnesium. This curve appeared to consist of more than one independent curve of activity. In fact, Gomori concluded that intestinal phosphatase consists of three components which occur in variable proportions in different enzyme preparations. Two of these components, having half-lives of ten minutes and two and one half hours respectively, are rapidly inactivated while acting on the substrate in the absence of magnesium. The third component is stable for several days. The activation of intestinal phosphatase by magnesium and alanine was also studied. The three components have different ratios of activation by magnesium of 20, 1.5, and 3 respectively. It was found that alanine had the greatest activating effect on old enzyme preparations which contained a greater amount of the stable component. The activating effect of alanine is thought to be due to a transformation of part of the stable component into one of the unstable but more active components. Thus it can readily be seen that the complex nature of alkaline phosphatase activity has not yet been elucidated.

Alkaline phosphatase activity is necessary for a variety of biochemical processes. For example, phosphatase is important in calcification; but its function in calcification has not yet been clearly defined. Robison (9,10) was the first to link phosphatase activity with calcification

when he discovered that the enzyme was especially abundant in ossifying cartilage. He proposed that the function of alkaline phosphatase was to increase the local concentration of inorganic phosphate by its action on phosphate esters. He predicted that the local phosphate and calcium concentrations would then be great enough to exceed the solubility product and bone salt would be deposited. Recent investigations have stressed the importance of the organic phosphate ester substrate as well as the hydrolytic product, inorganic phosphate. Neuman, DiStefano, and Mulryan (11) observed that ester phosphate is strongly adsorbed by bone mineral and in this way inhibits further calcification. Ester phosphate is also a calcium complexing agent. Phosphatase activity is therefore required to hydrolyze ester phosphate so that calcification can take place. Evidently alkaline phosphatase plays an important role in preparing tissues for calcification.

There is also an interdependence of alkaline phosphatase and vitamin D. Zetterström (12) found that alkaline phosphatase is concerned with the metabolic role of vitamin D in the prevention of rickets. He observed that water-soluble phosphorylated vitamin D₂ activated the alkaline phosphatase from kidney, intestine, and bone and he reasoned that the enzyme would appear to be essential for intestinal absorption of phosphate, for kidney tubule reabsorption of phosphate, and also for bone phosphate deposition.

A great deal of work has been done at the University of Alberta on the relation of diet to alkaline phosphatase. This aspect of the problem was studied by observing the effect of various dietary conditions on the level of serum alkaline phosphatase activity of rats. Serum enzyme activity, which decreases on fasting to a constant level, was found to be correlated with food consumption and in particular with the consumption of fat (13, 14). On the contrary, the protein content of the diet had no effect on the level of serum alkaline phosphatase (15). The effect of dietary oxalate on serum alkaline phosphatase levels has been investigated in Part I of this thesis. The elevated levels of serum phosphatase activity resulting from the ingestion of dietary fat appeared to be related to fat absorption from the intestine (16). Inhibition studies indicated that the increased phosphatase activity is derived largely from the intestine (17). Further investigations on intestinal alkaline phosphatase activity and fat absorption are contained in Part II of this thesis.

PART 1 : THE EFFECT OF DIETARY OXALATE ON RAT SERUM
ALKALINE PHOSPHATASE ACTIVITY

INTRODUCTION

Robison (9, 10) was the first to link bone alkaline phosphatase activity with endochondral calcification. The role of this enzyme in bone formation has been more clearly elucidated by the recent work of Follis (18), Neuman, DiStefano, and Mulryan (11), and Waldman (19). Follis also observed that in the rachitic rat the cartilage is rich in phosphatase.

Serum alkaline phosphatase activity is increased during the development of rickets and there has been speculation as to the source of the abnormal excess of the enzyme. Folley and Kay (20) have pointed out that the increased phosphatase activity associated with growing bone is reflected in high serum phosphatase levels in the young. In addition, Bodansky (21) has concluded that the levels of the serum enzyme in various diseases are associated with alterations in the alkaline phosphatase concentration in the organs affected. Motzok (22) has provided evidence that the elevated serum alkaline phosphatase level of rachitic chicks is of skeletal origin.

Recent work in this laboratory on the effect of dietary factors on serum alkaline phosphatase in rats (15,23,24) was

extended to include a study of the effect of dietary oxalate on the enzyme. The animals were fed diets containing oxalate as the sodium salt or as dried rhubarb, in which oxalic acid is present in high concentrations. It has been shown that oxalates in food interfere with assimilation of calcium (25, 26, 27). Low calcium retention by the animal body has been attributed to the oxalate content of dietary spinach (28, 29). In our experiments, the interference with calcium metabolism resulted in rickets and corresponding changes in the serum alkaline phosphatase.

METHODS

1. CALCIUM DETERMINATION

Calcium was determined by the standard method of Kramer and Tisdall (30) which involves titration with permanganate. The calcium contents of the diets and of a few of the carcasses were determined in this way.

2. OXALATE DETERMINATION

The oxalate content of the powdered rhubarb and of the diets containing oxalate was determined by the gravimetric method of Nelson and Mottern (31). Oxalate was weighed as calcium oxide.

3. LINE TEST

Tibiae sections were stained with 2% silver nitrate solution to determine the extent of calcification(32).

4. PHOSPHATASE DETERMINATION

Alkaline phosphatase levels were determined by the micromethod of Shinowara, Jones, and Reinhart (33) as modified by Gould and Schwachman (34). The unit of phosphatase activity is defined by Shinowara as "equivalent to one mgm. of phosphorus as phosphate ion liberated during one hour of incubation at 37°C. with a substrate containing sodium beta-glycerophosphate, hydrolysis not exceeding 10% of the substrate and at optimum pH of ... 9.3 ± 0.15 .

5. SERUM CALCIUM AND MAGNESIUM DETERMINATION

Serum calcium and magnesium were determined by a modification of the method of Herschfelder and Serles (35). The calcium was precipitated with oxalate and determined by titration with permanganate. The magnesium was determined colorimetrically in the supernatant fluid.

CALCIUM DETERMINATION

Reagents:-

3% sodium oxalate

2% ammonium hydroxide

1 N sulphuric acid

0.01 N potassium permanganate

Method:-

One ml. of serum was pipetted into a centrifuge tube. 0.5 ml. of 3% sodium oxalate and 1.5 ml. of water were added. This mixture was incubated in a 45°C. water bath for 20 minutes to accelerate the precipitation of calcium oxalate. The tubes were then centrifuged at 2000 r.p.m. for 15 minutes. The supernatant was set aside for the magnesium determination.

The precipitate of calcium oxalate in the centrifuge tube was drained for five minutes by inversion and then washed with 3 ml. of 2% ammonium hydroxide and centrifuged down again. This procedure of draining, washing, and centrifuging was repeated once. 2 ml. of 1 N sulphuric acid was added to the precipitate. The tubes were then placed in a boiling water bath until the precipitate was completely dissolved. The hot oxalate solution was titrated with 0.01 N potassium permanganate until a definite pink persisted for at least one minute. One ml. of 0.01 N potassium permanganate is equivalent to 0.2 mg. of calcium or 20 mg. of calcium per 100 ml. of serum.

MAGNESIUM DETERMINATION

In this determination the serum proteins are precipitated by means of trichloroacetic acid. Titan yellow is added to the resulting clear supernatant. On the addition of sodium hydroxide a red magnesium hydroxide-Titan yellow complex

is formed. Hydroxylamine hydrochloride stabilizes this color lake (36) which is dispersed by the colloid, starch.

Reagents:-

10% trichloroacetic acid

1.5 N sodium hydroxide

2% hydroxylamine hydrochloride

0.075% Titan yellow solution

stored in a dark bottle in the refrigerator.

1.5% starch solution

prepared daily and filtered with pyrex wool.

Standard Magnesium Solutions

Stock Solution:-

10.131 gm. of magnesium sulphate heptahydrate is dissolved in distilled water. Then 0.05 ml. of chloroform is added and the volume made up to one litre with distilled water. One ml. contains one mgm. magnesium.

Working Solution:-

2 ml. of the stock solution is diluted with distilled water to 100 ml. One ml. contains 0.02 mgm. magnesium.

Method:-

To 2 ml. of the calcium-free serum, 2 ml. of 10% trichloroacetic acid were added and the mixture was

allowed to stand for 5 minutes. The tube was centrifuged. 3 ml. of the clear supernatant was pipetted into a 15 x 150 mm. test tube. To this supernatant the following were added in the order named:- 0.9 ml. water, 0.6 ml. 1.5 N sodium hydroxide, 1 ml. hydroxylamine hydrochloride, 1.5 ml. starch solution, 1 ml. Titan yellow, and 2 ml. 1.5 N sodium hydroxide. The contents were mixed thoroughly after each addition. The resulting solution was allowed to stand for 10 minutes. Then the intensity of the red color was measured in a Coleman Universal Spectrophotometer (Model #14) at 540 millimicrons using 13 x 13 x 100 mm. matched square cuvettes. The blank was prepared by adding the following reagents to 4.5 ml. distilled water:- 1 ml. hydroxylamine hydrochloride, 1.5 ml. starch solution, 1 ml. Titan yellow, and 2 ml. sodium hydroxide. The magnesium concentrations (mgm. magnesium per 100 ml. of serum) were read from a calibration curve (Figure 1). This curve was prepared by plotting the magnesium concentrations of several dilutions of the working magnesium standard solution against their percent transmission.

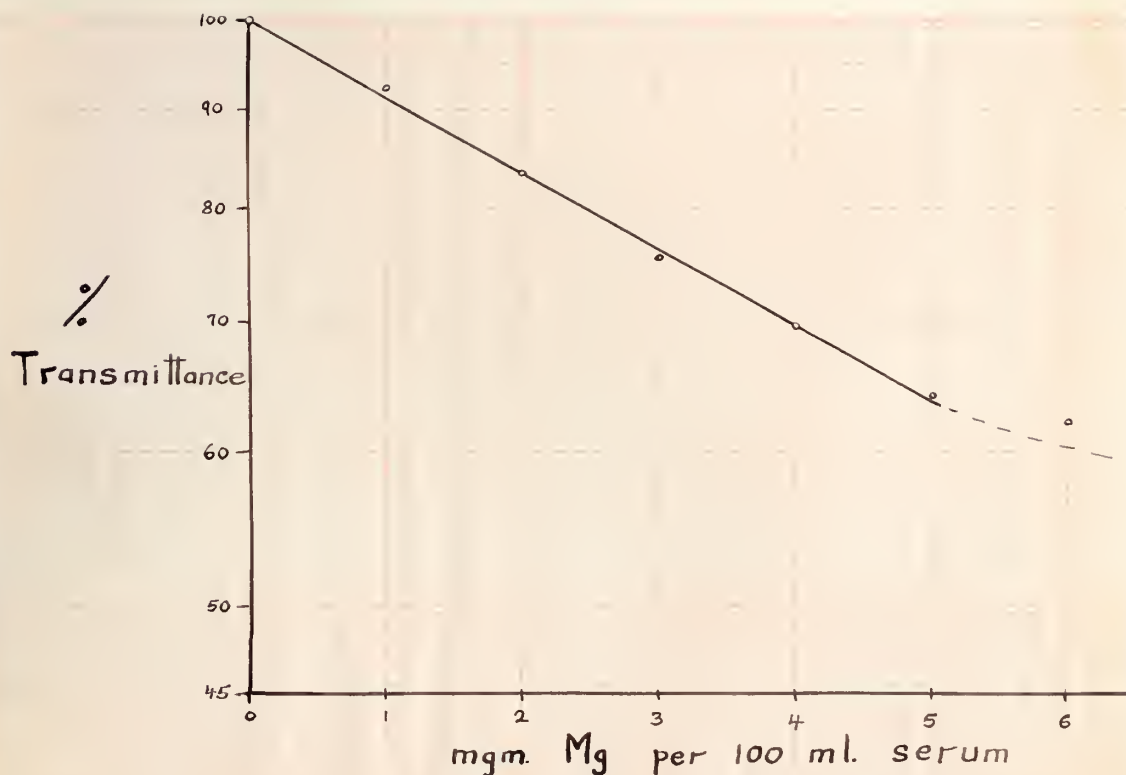
Table 1

The Relationship of Magnesium Concentration to Percent
Transmittance

Distilled Water ml.	Working Standard Mg Sol'n. ml.	mgm.Mg/ml. Colored Solution	mgm.Mg/ 100 ml. Serum	% Trans- mittance average reading
4.0	0.5	0.01	1.0	92.3
3.5	1.0	0.02	2.0	83.6
3.0	1.5	0.03	3.0	75.5
2.5	2.0	0.04	4.0	69.7
2.0	2.5	0.05	5.0	64.0
1.5	3.0	0.06	6.0	62.1
0.5	4.0	0.08	8.0	57.0

Figure 1

Calibration Curve for the Magnesium Determination



EXPERIMENTAL

Six-week old male albino rats were housed in individual cages and given food and water ad libitum. These rats were separated into four groups of six each so that the average weight per group was 133 gm. Each group was maintained on one of the four synthetic diets listed below:-

Diet 1 was a control diet which contained sufficient calcium for optimal growth (37). It contained 68.1% sucrose, 20% casein, 4% Crisco, a hydrogenated vegetable fat, 2 % cod liver oil, 3.2% calcium lactate, and 2.7% calcium-free McCollum's salt mixture. Each kilogram of diet also contained the following vitamins:- 10 mgm. thiamine hydrochloride, 10 mgm. pyridoxine hydrochloride, 10 mgm. niacin, 20 mgm. calcium pantothenate, 20 mgm. riboflavin, and 1 gm. choline chloride. The calcium content of the diet was determined to be 0.50%.

Diet 11 was identical with Diet 1, except that it had a very low calcium content, as a result of replacing the calcium lactate with sucrose. Analysis proved that this diet contained 0.09% calcium, present chiefly in the casein component.

Diet 111 contained rhubarb, a foodstuff high in oxalate. Dried powdered rhubarb petioles made up 15.4% of the diet, replacing an equal weight of sucrose. Analysis of the powdered rhubarb revealed that the oxalate content was 6.01% and the calcium content was 0.92%. Calcium lactate to the

extent of 1.4% was also added. Otherwise the diet was the same as Diet 1. Diet 111 contained 0.95% oxalate and 0.50% calcium.

Diet 1V contained oxalate equivalent to the content of Diet 111 but in the form of the sodium salt. Diet 1 was modified by replacing an equivalent amount of sucrose by 2.3% calcium lactate and 1.5% sodium oxalate. The concentrations of oxalate, 0.95% and of calcium, 0.50%, were equal to those of Diet 111.

The calcium and oxalate contents of the diets are tabulated as follows:-

Table 11

The Calcium and Oxalate Contents of the Diets

Diet	1	11	111	1V
% calcium	0.50	0.09	0.50	0.50
% oxalate	—	—	0.95	0.95

Assuming that oxalate present in Diets 111 and 1V combined with an equivalent amount of dietary calcium, it was estimated that there would be an excess of 0.07% calcium, approximately the amount present in Diet 11. An additional experiment on calcium retention in animals receiving dietary oxalate was carried out and is described below under Results.

Serum inorganic phosphorus and alkaline phosphatase levels were determined bi-weekly on samples of blood "milked" from the tails. The animals were killed by decapitation at the

end of six weeks. The blood from each animal was collected and determinations were made of the serum alkaline phosphatase level and of the serum phosphorus, calcium, and magnesium. The tibiae were removed from two animals in each group. One tibia from each of these animals was used to determine the extent of calcification by the line test method. The other tibia was ashed for eight hours at 750° C. and the weight of the ash was determined.

RESULTS

The serum alkaline phosphatase values at two-weekly intervals are given in Table III. Diet IV proved to be toxic after four weeks and only two animals survived the entire experimental period of six weeks. The average value for these two animals is included in the table, but without a standard error of the mean. The phosphatase values of the four groups at zero time are not significantly different from one another. An analysis of variance of the phosphatase values indicates that the values of the rats receiving Diet I are significantly lower than the values of the rats receiving the other three diets. The former values are in the normal range.

The increase in serum alkaline phosphatase activity of the animals subsisting on Diets II, III, and IV is emphasized in Table IV.

Table 111

Effect of Dietary Oxalate on Serum Alkaline Phosphatase
of Growing Rats

serum alkaline phosphatase expressed as units / 100 ml. serum

Diet	0 time	2 weeks	4 weeks	6 weeks
1	105 $\pm$ 4.4	119 $\pm$ 3.3	98 $\pm$ 4.4	102 $\pm$ 6.9
11	93 $\pm$ 1.9	158 $\pm$ 7.6	123 $\pm$ 9.1	127 $\pm$ 2.8
111	106 $\pm$ 6.2	144 $\pm$ 7.2	143 $\pm$ 5.4	133 $\pm$ 5.2
1V	109 $\pm$ 6.2	142 $\pm$ 5.2	125 $\pm$ 13.0	129*

each value is the mean of six rats $\pm$ standard error of mean

* average for two rats

Table 1V

Effect of Dietary Oxalate on Serum Alkaline Phosphatase
of Growing Rats

serum alkaline phosphatase as % of values at 0 time

Diet	0 time	2 weeks	4 weeks	6 weeks
1	100	113	93	97
11	100	170	132	137
111	100	136	135	125
1V	100	130	115	118

Table V

Effect of Dietary Oxalate on Body Weights (gm.) (W); Ash Content of Tibiae (gm.) (T); Serum Phosphorus (mgm.per 100 ml. serum) (P); Serum Calcium (mgm.per 100 ml. serum) (Ca); and Serum Magnesium (mgm.per 100 ml. serum) (Mg).

Diet	W	T	P	Ca	Mg
I	285 $\pm$ 6.5	0.22*	9.5 $\pm$ 0.2	12.6 $\pm$ 0.1	2.9 $\pm$ 0.04
II	225 $\pm$ 11.9	0.12*	11.0 $\pm$ 0.5	10.9 $\pm$ 0.1	3.7 $\pm$ 0.15
III	171 $\pm$ 5.9	0.08*	10.3 $\pm$ 0.4	10.3 $\pm$ 0.5	3.6 $\pm$ 0.08
IV	119*	0.11*	14.0*	7.2*	3.9*

each value is the mean of six rats($\pm$ standard error of the mean) except as noted

* average for two rats

The amount of calcium retained in the bodies* of the animals receiving the rhubarb Diet 111 was tested separately with 18 young male rats, which were divided into three groups, averaging 114 gm. for each group. One group was sacrificed at once, and after removal of the intestines the animals were ashed at approximately 750°C.. The amount of calcium for the animals in this group averaged 1.06 gm. The remaining two groups of rats were maintained on Diets I and 111 for seven weeks. The average terminal weights were 251 gm. (I) and 233 gm. (111). The calcium content of their carcasses was then determined as for the first group and the average values were 2.4 gm. and 1.28 gm. respectively. Hence the amount of calcium retained in the bodies of growing rats on an oxalate containing diet was reduced to a very low level(0.22 gm.) as compared to 1.43 gm. for those on a normal calcium diet. The retained calcium constituted 0.98% of the increase in body weight during the experiment for group I, and 0.20% for group 111.

The data of Table V indicate the effects of the four diets over the six week test period on the growth of the rats, the ash content of their tibiae, and the levels of serum phosphorus, calcium, and magnesium.

* The writer is indebted to Mr. K.A. Siluch for this information.

The animals grew at a slower rate on Diets II and III than on Diet I, while the animals on Diet IV lost weight during the experiment and four died after the fourth week. A paralysis was noted in the legs of all the rats in this group, similar to the effect reported by Schmidt and Greenberg (38) in the case of pigs which lacked dietary calcium. The toxic effect of the oxalate in Diet III was not so marked, presumably because it was not so readily assimilated. Analyses of variance were performed on the other data of the table. Statistically significant differences exist between the tibia ash (T) and serum magnesium (M) values of the rats subsisting on Diet I and the rats subsisting on the other three diets. Serum phosphorus and calcium values of groups III and IV differ significantly from those of group I. The ratios of serum calcium, phosphorus, and magnesium in all groups are similar to those reported by Brookfield (39). It may be further noted that the values for the ash content of the tibiae given in Table V constitute 55% (I), 48% (II), 40% (III), and 45% (IV) of the wet weight of the tibiae before ashing.

The data so far presented are indicative of rickets in the animals maintained on the low calcium diet and the two oxalate-containing diets, and this is further borne out by the line test of the tibiae. These bones were fully calcified in the animals receiving adequate dietary calcium. Uncalcified zones in the epiphyseal region were detected in

the bones from the other three groups and this was especially marked with diets lll and lV.

DISCUSSION

The results presented above appear to justify the conclusion that rickets was produced in growing rats by maintaining them on low dietary calcium or by reducing the available dietary calcium by including oxalate in the regimen. Oxalate in common foods such as rhubarb may not be quite as effective as an equivalent amount of sodium oxalate added to the diet, depending on the amount which can be readily assimilated from the gastrointestinal tract. It would seem that diets containing oxalate in appreciable amounts may, under certain conditions, markedly affect the assimilation of dietary calcium.

Cantor, Wight, and Tuba (13) and Tuba and Madsen (14) have presented evidence for a positive correlation between food consumption and the levels of serum alkaline phosphatase. A significant correlation was found to exist between the levels of the serum enzyme and the daily food consumption of the animals maintained on the normal control Diet 1. The value of r is 0.925. In the case of the other three groups on Diets ll, lll, and lV no correlation was found between food consumption and serum phosphatase activity.

The consumption of these groups was below normal. The increase in serum alkaline phosphatase found in the latter three groups of rats, has been demonstrated previously to be a characteristic feature of rickets (40). It has been indicated that the increase in serum phosphatase is contributed by the bone (41).

The greatest increase in serum phosphatase, demonstrated in this experiment was found to occur when the rats were maintained on Diets ll, lll, and lV for two weeks. This effect shows up clearly in Table lV in which the serum enzyme levels of each group are based on 0 time values of 100%. Dikshit and Patwardhan (42) similarly found that the serum alkaline phosphatase levels of young rats fed rachitogenic diets were high at the beginning of the feeding period but decreased markedly as rickets developed. From this evidence they concluded that in rickets the increase in serum alkaline phosphatase may not necessarily originate from the bone.

In conjunction with recent work in this laboratory on the source of the serum alkaline phosphatase in rats (17), an inhibition study was carried out with the sera of animals subsisting on Diet lll midway through the fifth week of the experiment. Blood was obtained from the animals after a three-day starvation period which minimizes the contribution of the intestinal mucosa to the total alkaline phosphatase of the serum. The fasting levels of alkaline phosphatase for this group of animals averaged 49 units per 100 ml. serum,

which is significantly higher than the average value of 30 units obtained by Cantor, Wight, and Tuba (13) for rats fasted for three days after being maintained on a stock laboratory diet. Sodium taurocholate was found to inhibit bone phosphatase more than liver or intestinal phosphatase. The inhibition by 0.001 M sodium taurocholate of the serum phosphatase of the fasted rachitic rats of group III was greater than the inhibition of the serum phosphatase of fasted normal rats. This suggests that the increased phosphatase activity associated with rickets may be derived from the bone.

Motzok (22) has recently presented evidence that the increased plasma alkaline phosphatase of rachitic chicks is of skeletal origin. He studied the effects of vitamin D on plasma and tissue phosphatase activities. The phosphatase activities of bone and plasma were markedly influenced by vitamin D in the diet whereas the phosphatase activities of intestinal mucosa, kidney, and liver are not related to the vitamin D content of the diet.

The evidence presented above seems to suggest that the increased serum alkaline phosphatase of rachitic rats may be derived from the bone.

SUMMARY

1. Increased levels of serum alkaline phosphatase were produced in growing rats by diets low in calcium or by the addition of sodium oxalate or rhubarb to diets containing adequate amounts of calcium.
2. Variations from normal in levels of serum phosphorus, calcium, and magnesium, as well as in the ash of tibiae, indicate that the animals were rachitic.
3. The amount of calcium retained in the bodies of animals maintained on a diet containing rhubarb, which has a high oxalate content, were very much lower than in growing rats fed a normal calcium diet.
4. The source of the increased serum alkaline phosphatase activity associated with rickets is probably the bone.

PART 11 : INTESTINAL PHOSPHATASE ACTIVITY
 AND FAT ABSORPTION

INTRODUCTION

Alkaline phosphatase activity has been repeatedly associated with fat metabolism. There is a highly significant correlation between serum alkaline phosphatase activity and fat consumption (13, 14) and the high serum alkaline phosphatase levels of alloxan diabetic animals are related to increased utilization of fat (13). Weil and Russell (43) found that it was the alcohol-ether soluble fraction of the diet which elevated the low plasma alkaline phosphatase level of fasting and that the effective components were certain unsaturated fatty acids and cephalin. From their experimental results, they concluded that the increased serum alkaline phosphatase activity associated with the absorption of certain fatty acids was related to a phosphorylation-dephosphorylation mechanism for fat absorption. They thought it possible that fatty acids could be transformed into phospholipids in the intestine. Phosphatase activity would be required for resynthesis of neutral fat.

When fat is ingested, intestinal phosphatase activity also exhibits an elevation which parallels that of the serum phosphatase activity. Flock and Bollman (44) showed that the phosphatase activity of rat intestinal lymph is decreased with fasting and is increased with fat absorption but to a greater extent than plasma phosphatase activity. They found that the serum phosphatase level could be reduced by preventing the lymph from reaching the blood. They arrived at the conclusion that the increased serum phosphatase activity resulting from fat ingestion is supplied by the intestine. Inhibition studies performed in this laboratory (17) provide further evidence that the intestine, which has a very high concentration of phosphatase, is the source of increased serum phosphatase activity associated with fat absorption.

The mechanism of fat absorption from the intestine has not yet been elucidated. The following series of experiments, which are listed below, were planned to investigate the effect of fat ingestion on intestinal alkaline phosphatase activity.

Experiment #1 - The Response of Intestinal Alkaline Phosphatase of Fasted Rats to Force-Fed Fat.

Experiment #2 - The Response of Intestinal Alkaline Phosphatase of Fasted Rats to the Addition of Choline to Force-Fed Fat.

Experiment #3 - The Response of Intestinal Alkaline Phosphatase of Fasted Rats to Fatty Acid Ingestion.

METHODS

1. THE EXPERIMENTAL ANIMAL

Adult male albino rats were housed in individual cages. They were maintained on a diet of Purina fox checkers and water ad libitum for at least three days before each experiment and an adequate supply of water was always available during fasting periods.

In order to determine serum and intestinal alkaline phosphatase activity the animals were killed by decapitation. The serum was separated at once and stored in the refrigerator. The first ten cm. of the intestine from the pylorus was removed and kept on ice until it was washed with cold distilled water. It was blotted dry, weighed, and then homogenized with cold distilled water in a Potter glass homogenizer. The concentrated homogenate was stored overnight in the refrigerator in a 250 ml. volumetric flask. The following morning each homogenate was made up to the required volume and shaken. Part of the homogenate was centrifuged and an aliquot of the supernatant was diluted ten times for the determination of phosphatase activity.

2. PHOSPHATASE DETERMINATION

The alkaline phosphatase activities of the serum and intestine were determined by a macro-method. This method is based on the micro-method of Shinowara, Jones, and Reinhart (33) as modified by Gould and Shwachman (34).

The unit of phosphatase activity is defined by Shinowara as
 "equivalent to one mg. of phosphorus as phosphate ion
 liberated during one hour of actual incubation at 37°C. with
 a substrate containing sodium beta-glycerophosphate
at optimum pH of 9.3 ± 0.15 ."

Reagents:-

Substrate-Buffer Mixture

0.4240 gm. sodium diethyl barbiturate
 0.5000 gm. sodium beta-glycerophosphate
 0.2464 gm. magnesium sulphate heptahydrate
 made up to 100 ml. with distilled water.

10% trichloroacetic acid

0.1 N sodium hydroxide

Molybdic Acid

Stock Solutions:-

7.5% sodium molybdate
 10 N sulphuric acid

Working Solution:-

consists of one part 7.5% sodium molybdate to one part
 10 N sulphuric acid and is prepared immediately before use.

Stannous Chloride

Stock Solution:-

6 gm. stannous chloride
 10 ml. concentrated hydrochloric acid
 covered with a toluene layer to prevent oxidation and
 stored in a dark bottle in the refrigerator.

Working Solution:-

0.2 ml. of the stock solution is diluted to 100 ml.

kept cold and used within four hours of preparation.

Standard Phosphorus Solutions

Stock Solution:-

0.4394 gm. potassium acid phosphate is made up to one litre with distilled water. One ml. contains one mg. phosphorus.

Working Solution:-

2 ml. of the stock solution is diluted to 100 ml.

One ml. contains two micrograms phosphorus.

Method:-

Serum dilutions of 1:10 and 1:100 were prepared for determining the serum inorganic phosphorus content and the alkaline phosphatase activity respectively. A 1:10 dilution of intestinal homogenate was used for determining both the inorganic phosphorus content and the alkaline phosphatase activity of the intestine. 0.5 ml. of diluted serum or intestinal homogenate was pipetted into a 13 x 100 mm. test tube which was placed in a 37°C. water bath. 1.0 ml. of substrate-buffer mixture was added and the resulting mixture incubated at 37°C. for exactly one hour. For the corresponding determination of inorganic phosphorus 1.0 ml. of water was added rather than 1.0 ml. of the substrate-buffer mixture. At this stage 1.0 ml. of 10% trichloroacetic

acid was added to stop enzyme activity and precipitate protein. The tubes were then centrifuged.

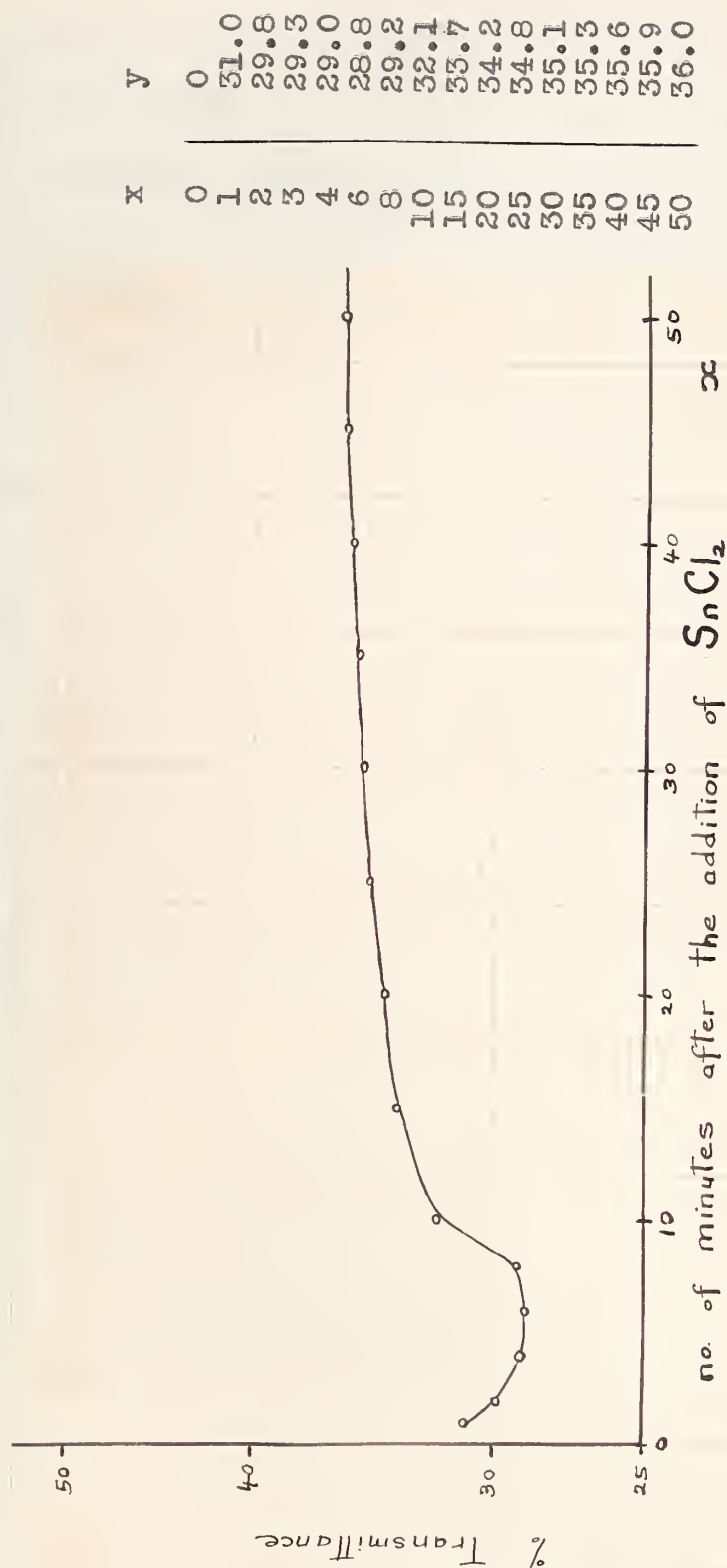
For color development 1.5 ml. of the supernatant was pipetted into a 15 x 120 mm. test tube. 2.1 ml. of 0.1 N sodium hydroxide, 1.2 ml. molybdic acid, and 1.2 ml. stannous chloride were added in that order. The contents were mixed thoroughly after each addition. At the end of 23 minutes after the addition of stannous chloride* the color intensity was measured in a Coleman Universal Spectrophotometer (Model #14) at 600 millimicrons, using 13 x 13 x 100 mm. matched square cuvettes. The required blank was prepared with 3.6 ml. distilled water. 1.2 ml. molybdic acid and 1.2 ml. stannous chloride were added.

*Note on Color Development

The blue color of reduced oxides of molybdenum that develops with the addition of stannous chloride is unstable. As a special precaution it was necessary to standardize the period of time allowed from the addition of stannous chloride to the measurement of the intensity of the color in the spectrophotometer. This was done by reading the percent transmittance of a sample at one minute intervals after the addition of stannous chloride. The readings were recorded in Figure 2. From this graph it was decided to measure the color intensity 23 minutes after the addition of stannous chloride.

Figure 2

The Relationship of Percent Transmittance to Time Allowed for Color Development



The phosphorus concentrations were read from a calibration curve (Figure 3). This curve was prepared by plotting the phosphorus concentrations of several dilutions of the working phosphorus standard solution against their percent transmittance. The most suitable formula of the linear regression calibration curve was found to be

$$X = 70.69 - (35.46 \times \log \% \text{ transmittance})$$

where X is mgm. phosphorus per 100 ml.

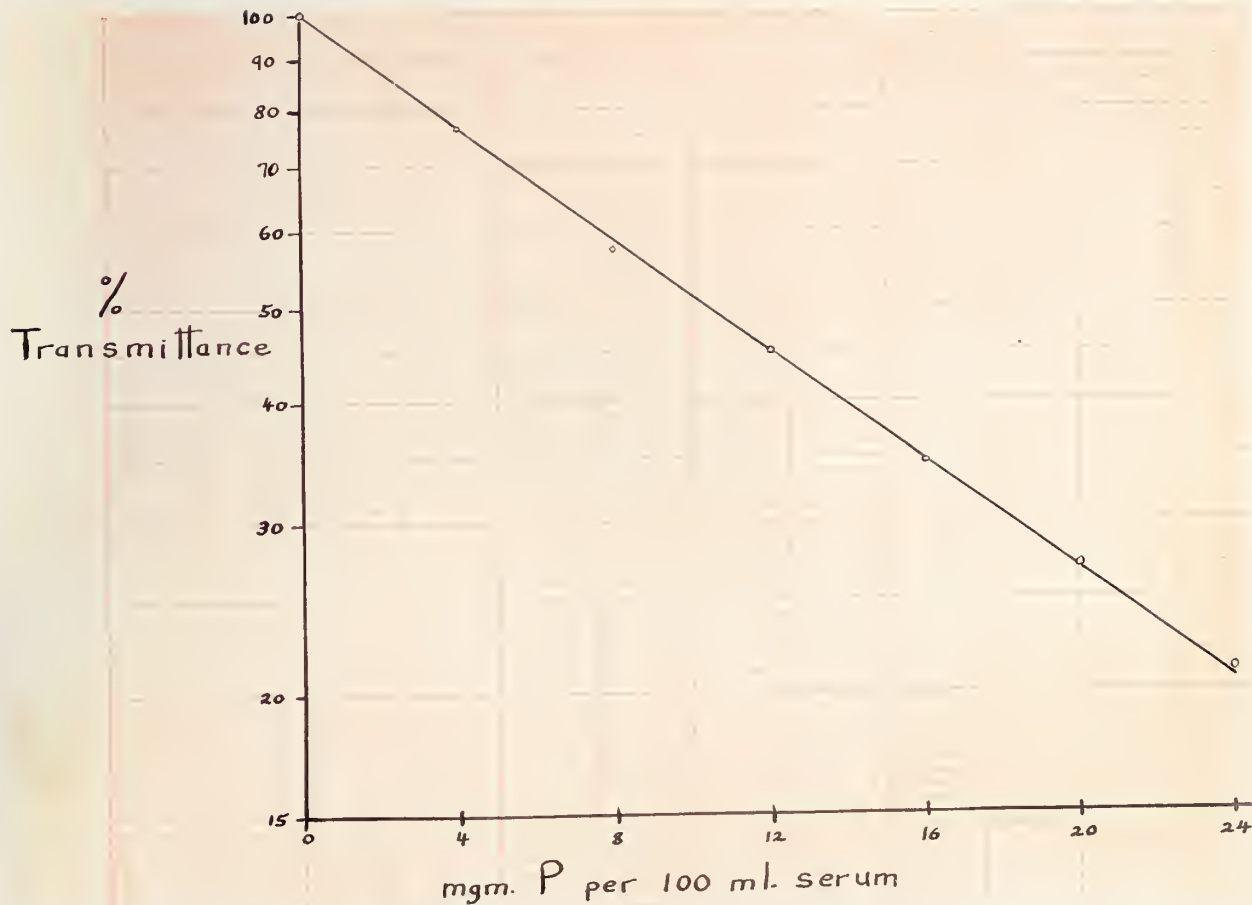
Table VI

The Relationship of Phosphorus Concentration to Percent Transmittance

Distilled Water ml.	Working Standard P Sol'n. ml.	Microgm. P per ml. Colored Solution	mgm. P / 100 ml. Serum	% Transmittance average reading
3.6	0.0	0.0	0	100.0
3.0	0.6	1.2	4	76.5
2.4	1.2	2.4	8	57.2
1.8	1.8	3.6	12	45.0
1.2	2.4	4.8	16	34.7
0.6	3.0	6.0	20	27.0
0.0	3.6	7.2	24	21.0

Figure 3

Calibration Curve for the Phosphorus Determination



0
x
t
h
y
v

2
e
a
d
e
f
g

7
z
y
w
v
u

h
q
p
o
n
m

EXPERIMENT #1
THE RESPONSE OF INTESTINAL ALKALINE PHOSPHATASE
OF FASTED RATS TO FORCE-FED FAT

INTRODUCTION

The levels of intestinal and serum alkaline phosphatase are significantly decreased in fasted rats (13,17,43,44) and there is an increase in the activity of both enzymes following the feeding of fat (13,45,46,47). It has been demonstrated that the increased amounts of the serum enzyme, associated with the ingestion of fat, are derived from the intestine (17, 44).

The nature of protein in the diet, when supplied in adequate amounts, does not affect the serum alkaline phosphatase levels of growing or adult rats (15). However, protein depletion decreases the activity of the enzyme in the liver (48-50) and small intestine (51) of adult rats. The following experiment was designed to determine the ability of the intestine to synthesize alkaline phosphatase in rats which had been subjected to protein depletion by fasting for 2,6, or 12 days and then force-fed fat.

EXPERIMENTAL

A preliminary experiment was carried out to establish the optimum time after force feeding for determination of serum and intestinal alkaline phosphatase levels. Rats were fasted in four groups of three for one, two, four, and six days respectively. At the termination of a fast the animals were force-fed one ml. of olive oil, or one ml. of water in the case of the controls, by blowing the liquid through a pipette directly into the esophagus. In each group of three a rat was killed at the end of each interval of four hours after force feeding. The serum was collected, intestinal homogenates were prepared, and serum and intestinal alkaline phosphatase levels were determined by the method outlined previously. This preliminary experiment indicated that the optimum time for obtaining the maximum effect of olive oil on the activity of serum and intestinal alkaline phosphatase was eight hours after force feeding. There are diurnal variations in the levels of both the serum and intestinal enzyme and for this reason it was decided to kill the animals at 4 P.M..

Four groups of animals were selected and they were paired within groups according to body weight at the beginning of the experiment. One group of animals (Group 1) was maintained on the regular laboratory diet, while the remaining three groups were fasted for 2, 6, and 12 days

respectively. On the final day the animals were weighed at 8 A.M. and in the fasted groups one of the paired rats was fed 1 ml. water and the other animal was given 1 ml. olive oil. Eight hours later all the animals were decapitated. Serum was collected and the usual portion of the small intestine was homogenized as before. Each liver was extirpated and homogenized in a Waring blender, and the homogenate was made up to 200 ml. with water. A 10 ml. aliquot of each liver homogenate was used for a macro-Kjeldahl determination of the nitrogen content. The enzyme levels in the intestine and serum, and the nitrogen content of the liver were determined for each animal. The values of the liver nitrogen and intestinal alkaline phosphatase are expressed on the basis of 100 gm. initial body weight (48). In this way it is possible to compare the data obtained from animals fasted for different periods and to allow for the weight losses undergone by the tissues.

RESULTS

The data of Table VII indicate that the values of intestinal and serum alkaline phosphatase fell sharply as a result of a two day fast and continued at approximately the same low levels through more extended periods of food deprivation. The greatest fall in liver nitrogen occurred during the first two days of fasting, but a fairly steady

decline in protein levels continued when the rats were fasted for six and twelve day periods. The liver protein values are significantly different for the animals fasted for 0, 2, 6, and 12 days. The loss of liver protein in the fasting rats is paralleled by the decreased protein content of homogenates of whole intestine (the first 10 cm. of the intestine from the pylorus). The latter data are not indicated in Table VII. The mean intestinal nitrogen (mgm. per 100 gm. initial body weight) for groups of three animals fasted for 0, 2, 6, and 12 days respectively were; 7.95 ± 0.37 ; 6.98 ± 0.38 ; 5.70 ± 0.11 ; 5.37 ± 0.07 .

The results indicate that serum alkaline phosphatase levels were elevated in all three fasted groups following the force-feeding of olive oil. However, the response was progressively lessened with longer periods of fasting. The group fasted for two days showed approximately 100 per cent elevation of intestinal phosphatase activity following the ingestion of olive oil. On the other hand, the groups fasted for 6 and 12 days manifested exactly the opposite response to the force-fed fat.

Table VII

Effect of Olive Oil on Alkaline Phosphatase Levels of Serum and Intestine of Rats Fasted
for Various Periods of Time

Fasting Period in days	Substance Force-fed	Body Weight in gm.		Liver Nitrogen in mgm. / 100 gm. initial body weight	Intestinal Phosphatase in units / 100 gm. initial body weight	Serum Phosphatase in units / 100 ml.
		Initial	Final			
0		242 (6)		121.1 $\pm$ 3.9 (6)	1014 $\pm$ 57 (6)	88.7 $\pm$ 8.1 (6)
2	water	294 (14)	261 (14)	82.8 $\pm$ 1.1 (14)	683 $\pm$ 77 (3)	22.5 $\pm$ 1.1 (3)
	olive oil				1238 $\pm$ 340 (3)	97.6 $\pm$ 2.1 (3)
6	water	305 (24)	226 (24)	65.7 $\pm$ 1.4 (24)	540 $\pm$ 30 (6)	30.8 $\pm$ 3.3 (6)
	olive oil				323 $\pm$ 34 (6)	70.9 $\pm$ 10.5 (6)
12	water	283 (20)	175 (20)	51.5 $\pm$ 1.7 (20)	549 $\pm$ 19 (6)	24.6 $\pm$ 1.8 (6)
	olive oil				277 $\pm$ 18 (6)	54.5 $\pm$ 4.6 (6)

Data are means ($\pm$ standard error of mean) of the number / group bracketed under mean value

DISCUSSION

It has again been demonstrated that rat serum alkaline phosphatase is composed of one fraction which is unaffected by fasting and a second or larger fraction which responds relatively quickly to fasting and the ingestion of fat. This variable or adaptive fraction appears to be largely derived from the intestine (17), where it seems to be essential in the absorption of fat (13,16,45,46,47). Intestinal alkaline phosphatase also is made up of a part which varies with the diet and nutritive state, as well as a non-adaptive part which remains reasonably constant even after periods of prolonged fasting up to 12 days. These constant reserves of the enzyme appear to assist in the absorption of fats, since in our experiments ingestion of fat is always followed by increased serum phosphatase levels. This result is consistent with the work of Flock and Bollman (47) who found that fat and phosphatase are removed from the intestine by the lymphatic system. In the case of the animals which were fasted for two days only, ingestion of fat appears to be a stimulus for the synthesis of alkaline phosphatase over and above amounts required for maintenance of the basic reserves in the intestine. Animals fasted for a longer time have had their protein reserves of liver and intestine depleted. As a consequence of this

excessive depletion they have lost the ability to synthesize sufficient enzyme to replace even that portion of the non-adaptive enzyme which has been translocated to the plasma by way of the lymph during fat absorption. This is in agreement with the previous work of Miller (48) who found that the concentrations of five liver enzymes, including alkaline phosphatase, were lowered in animals subjected to protein deprivation. Lawrie and Yudkin (51) noted that inadequate dietary protein intake was reflected in lowered concentration of intestinal alkaline phosphatase.

SUMMARY

1. Rat intestinal alkaline phosphatase consists of an adaptive portion which varies with the dietary state, and a non-adaptive fraction which remains constant even during prolonged fasting.
2. The adaptive enzyme is probably translocated, during fat absorption, by the lymphatic route and thus serves to elevate serum levels of alkaline phosphatase.
3. Protein reserves in the liver and intestine can be lowered by prolonged fasting to the extent that the ingestion of fat, the normal stimulus for further synthesis of the intestinal enzyme, cannot produce complete replacement of the non-adaptive enzyme which has been lost to the serum.

EXPERIMENT #2

THE RESPONSE OF INTESTINAL ALKALINE PHOSPHATASE
OF FASTED RATS TO THE ADDITION OF CHOLINE TO FORCE-FED FAT

INTRODUCTION

Artom and Cornatzer (52) have revealed that both choline and fat are involved in phospholipid formation in the intestine. They have presented experimental evidence which indicates that "the supply of choline (or choline precursors) may represent a limiting factor for the formation of phospholipids during the absorption of fat from the intestine."

The intestinal mucosa is very active in phospholipid formation. Verzář (53) recognized the importance of phosphorylation in fat absorption when he found that feeding glycerophosphate accelerated fat absorption from the intestine. In the course of later work with radioactive phosphorus, Schmidt-Nielsen (54) found that phospholipid synthesis is increased in the intestine when oleic acid is being absorbed. However, more recently, Zilversmit, Chaikoff, and Entenman (55) came to the conclusion that phospholipid formation is not essential for fat absorption. They showed that the turnover rate of intestinal phospholipid was not necessarily greater during fat absorption than during the fasting state.

There appears to be a possibility from our work that intestinal phosphatase plays a role in the dephosphorylation of phospholipids. The following experiment was performed to ascertain if feeding choline with olive oil would result in any additional effect on intestinal phosphatase activity.

EXPERIMENTAL

Three groups of six rats each were used for this experiment. Each group was fasted for a four day period and the following substances were force-fed:-

2 ml. of water to the rats of the control group I,
1 ml. olive oil followed by 1 ml. water to group II,
1 ml. olive oil followed by 1 ml. 0.5% choline chloride to group III.

Four hours after feeding each rat was decapitated and the serum was collected. The first ten cm. of the intestine from the pylorus was removed and the intestinal homogenates were prepared in the usual way. Then the alkaline phosphatase activities of the serum and intestine were determined. The micro-Kjeldahl method was used to determine the nitrogen content of the intestinal homogenate supernatant used in the phosphatase determination. The alkaline phosphatase activity of the intestine was expressed in terms of units per 100 gm. wet weight of intestine and in units per 100 mg. intestinal homogenate supernatant nitrogen.

RESULTS

The alkaline phosphatase levels of the serum and intestine are tabulated below (table VIII). The low phosphatase values obtained with the rats of group I are typical of starvation levels. The data of groups II and III indicate that force feeding of either olive oil or olive oil and choline results in a marked elevation of the alkaline phosphatase activity of both serum and intestine. Statistical analysis showed that the serum and intestinal alkaline phosphatase levels of groups II and III were elevated significantly above the levels of group I.

When choline was administered along with the olive oil there was a noticeable increase in serum and intestinal phosphatase activity over values obtained with olive oil alone. This increase showed up statistically only in the case of intestinal phosphatase values which were expressed as units per 100 mgm. of intestinal nitrogen.

Table VIll

The Response of Serum and Intestinal Alkaline Phosphatase Levels of Fasted Rats
to Olive Oil and Choline

Group	Substance Force-fed	Intestinal Phosphatase units / 100 gm. wet weight of intestine	Intestinal Phosphatase units / 100 mgm. intestinal nitrogen	Serum Phosphatase units / 100 ml.
I	water	3708 $\pm$ 558	85.5 $\pm$ 12.3	38.08 $\pm$ 10.75
II	olive oil and water	5869 $\pm$ 576	146.1 $\pm$ 12.0	54.91 $\pm$ 5.88
III	olive oil and choline	6829 $\pm$ 710	186.5 $\pm$ 13.2	59.37 $\pm$ 5.76

each value is the mean of the readings for 6 rats $\pm$ standard error of the mean

DISCUSSION

The results of this experiment show a trend toward increased intestinal alkaline phosphatase activity when choline is fed along with the olive oil. A greater increase may show up by incorporating the choline in a diet rather than force feeding it. Artom and Cornatzer (52) observed that the radioactive phosphorus content of the intestinal phospholipids was considerably greater when choline and oil were given simultaneously than when oil was given alone. These observations support the suggestion that a phosphorylation-dephosphorylation mechanism plays a role in fat absorption from the intestine. The increased intestinal phosphatase activity with choline is probably related to the dephosphorylation of phospholipids.

An experiment of Frazer (56) is of interest in relation to the possible role of choline in the absorption of fat from the intestine. He observed that when rats were fed with olive oil alone, the intestinal cells were filled with large globules of fat. However, when choline was fed along with the olive oil, the intestinal cells were cleared of fat more rapidly. This observation would seem to support the suggestion that choline is a limiting factor for phospholipid synthesis during fat absorption from the intestine.

SUMMARY

1. Force feeding olive oil results in increasing the intestinal phosphatase level above the low fasting level.
2. This intestinal phosphatase response is augmented further when choline is force-fed along with the olive oil.
3. The results from this experiment appear to support the idea of phospholipid synthesis as a step in fat absorption from the intestine.

EXPERIMENT #3
THE RESPONSE OF INTESTINAL ALKALINE PHOSPHATASE
OF FASTED RATS TO FATTY ACID INGESTION

INTRODUCTION

Weil and Russell (43) showed that the ingestion of the unsaturated fatty acids (oleic, erucic, linoleic, and linolenic acids) caused an elevation of the fasting level of plasma phosphatase activity. A free carboxyl group appeared to be essential as oleyl alcohol produced no effect on enzyme activity. However neither saturated fatty acids nor short chain fatty acids were effective in elevating plasma phosphatase levels. This work suggests that different fatty acids are utilized by different mechanisms. More recently, as a result of their work with radioactive fatty acids, Kiyasu, Bloom, and Chaikoff (57) concluded that short chain fatty acids are transported from the intestine mainly by the portal pathway, whereas long chain saturated fatty acids are transported by the lymph pathway.

In previous experiments (pages 33, 40) we found that olive oil was a stimulus to increased intestinal phosphatase activity. The effect of the ingestion of different fatty acids on intestinal alkaline phosphatase activity is not known. The following experiment was planned to observe intestinal response to the feeding of three types of fatty

acids, namely:-

1. a short chain fatty acid - butyric acid
2. a long chain unsaturated fatty acid - oleic acid
3. a long chain saturated fatty acid - stearic acid.

EXPERIMENTAL

Force-feeding certain fatty acids to rats caused abnormally distended stomachs. To obviate this effect, each fatty acid was mixed with casein, which was selected because it has little effect on alkaline phosphatase activity (15). The mixture was placed in a food-tin and left with the rat overnight. In each case the diet was eaten by the following morning.

Sixteen rats were divided into four groups of four rats each and the experiment was carried out with each group separately. Each group of four rats was fasted for four days. At 5 P.M. on the fourth day of fasting, each rat of the group was given a different diet. The diets were as follows:-

Diet A - a control diet of 5 gm. of casein

Diet B - 4.97 gm. casein + 0.03 gm. butyric acid

Diet C - 4.9 gm. casein + 0.1 gm. oleic acid

Diet D - 4.9 gm. casein + 0.1 gm. stearic acid.

Diets B, C, and D contained 0.00035 gram-moles of fatty acid.

The rats were decapitated 24 hours after the diet was placed in the cage. The serum was collected, part of the intestine was removed in the usual way, and the alkaline phosphatase activity of serum and intestine was determined.

RESULTS

The results are summarized in Tables IX and X.

Table IX

Effect of Fatty Acid Ingestion
on Serum and Intestinal Phosphatase Levels of Fasted Rats

Diet	Serum Phosphatase units / 100 ml.	Intestinal Phosphatase units / 100 gm. wet weight of intestine
A	32.69 $\pm$ 3.30	7,478 $\pm$ 1,176
B	57.73 $\pm$ 6.62	12,066 $\pm$ 2,873
C	67.52 $\pm$ 8.96	14,798 $\pm$ 4,644
D	52.09 $\pm$ 8.45	10,643 $\pm$ 2,828

each value is the mean of the readings for 4 rats

$\pm$ standard error of the mean

Table X

Effect of Fatty Acid Ingestion

on Serum and Intestinal Phosphatase Levels of Individual Fasted Rats

Group Diet	Serum Phosphatase units / 100 ml.				Intestinal Phosphatase units / 100 gm. wet weight of intestine			
	I	II	III	IV	I	II	III	IV
A	32.32	37.12	23.39	37.91	5536	6682	6793	10902
B	74.70	53.75	43.27	59.19	9460	9135	8991	20679
C	88.33	70.72	44.44	66.60	10570	11011	8942	28667
D	51.43	68.12	28.69	60.13	8567	6750	8212	19044

Table IX shows that the ingestion of butyric acid, oleic acid, and stearic acid of Diets B, C, and D respectively resulted in serum and intestinal alkaline phosphatase levels which were higher than those resulting from the ingestion of the control Diet A. A statistical analysis of variance showed that the serum phosphatase levels of the rats which were fed Diets B, C, and D were elevated significantly above the level of the rats fed the control Diet A. An analysis of variance of intestinal phosphatase levels showed that the levels after ingestion of Diets B and C were significantly higher than the level of the rats fed Diet A. The ingestion of oleic acid had the greatest effect on both the serum and intestinal phosphatase levels. The lower values obtained with stearic acid may be due to slower absorption of stearic acid. In previous work done in this laboratory, Taylor, Madsen, and Tuba (16) found that stearic acid had a delayed effect on serum phosphatase activity.

Table X indicates that phosphatase activity is subject to variation between groups. Variation between groups is undoubtedly associated with the time taken by the individual animals to consume their food. The trend of elevation of intestinal phosphatase activity with ingestion of butyric acid, oleic acid, and stearic acid shows up more markedly when each group is considered separately as in Table X than in Table IX where the average values are given.

DISCUSSION

The results of this experiment provide evidence that intestinal alkaline phosphatase activity plays a role in fatty acid absorption. The pronounced effect of oleic acid in elevating serum phosphatase activity agrees with the observations of Weil and Russell (43) and of Taylor, Madsen, and Tuba (16). There is a corresponding two-fold increase of intestinal phosphatase activity over that obtained with the control group which was fed casein alone. This intestinal response supports the conclusion that the intestine is the source of increased serum alkaline phosphatase activity after fat ingestion.

Weil and Russell had found that short chain fatty acids did not affect plasma phosphatase activity. However, in this experiment, ingestion of butyric acid did produce an elevation of the level of both serum and intestinal alkaline phosphatase activity. The effect of butyric acid was not as great as that of oleic acid.

There was a small increase in phosphatase activity when stearic acid was fed. In a dietary study, Taylor et al observed that stearic acid increased serum alkaline phosphatase activity after a period of one week on the diet. This delayed response to stearic acid ingestion may explain the negative result of Weil and Russell's short-term experiment.

The observation of increased alkaline phosphatase activity with the ingestion of both long and short chain fatty acids would seem to suggest that the absorption mechanism might be the same for all types of fatty acids. The effect of the three types of fatty acids in increasing serum and intestinal alkaline phosphatase activity emphasizes the role of phosphatase in fatty acid absorption.

SUMMARY

1. The ingestion of fatty acids results in an elevation of both serum and intestinal alkaline phosphatase activity.
2. Three types of fatty acids were tested, all of which elevated the alkaline phosphatase levels. The most pronounced effect was produced by the ingestion of the long chain unsaturated fatty acid, oleic acid. The short chain fatty acid, butyric acid, and the long chain saturated fatty acid, stearic acid, also raised the serum and intestinal phosphatase levels, the former being more effective than the latter.
3. These results suggest that intestinal alkaline phosphatase plays a role in the absorption of fatty acids.

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